PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/001239	11.02.2004	03.04.2003

International Patent Classification (IPC) or both national classification and IPC
C12P19/32, C12P19/40, C07H19/16, C07H19/20

Applicant
PRO.BIO.SINT.S.P.A.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/001239

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was field, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2004/001239

1) Reference is made to the following documents:

D1: GB-A-2 006 185 (AJINOMOTO KK) 2 May 1979 (1979-05-02)
D2: US-A-5 602 246 (BAUMAN JOHN G ET AL) 11 February 1997 (1997-02-11)
D3: WO 95/09244 A (SCHERING AG ;HUMMEL MARQUARDT HEIDI (DE); SCHMITZ THOMAS (DE); KEN) 6 April 1995 (1995-04-06)
D4: EP-A-0 376 518 (LILLY CO ELI) 4 July 1990 (1990-07-04)
D5: US-A-6 046 322 (TILSTAM ULF ET AL) 4 April 2000 (2000-04-04)

2) Claims 1-7:

The subject-matter of claims 1-7 concerns a process for the preparation of fludarabine phosphate wherein:
- in step a) 2-fluoroadenine is reacted with Ara-U in the presence of *Enterobacter aerogenes* to give crude fludarabine,
- in step b) and c) the crude fludarabine is purified by acetylation and crystallization in organic solvents and water,
- in step d) phosphorylation of pure fludarabine according to any conventional technical to give fludarabine phosphate.

D1, which is considered to represent the closest prior art, discloses a process for producing 9(β-D-arabinofuranosyl)purine which is optionally substituted in the 2,6- and/or 8-position (such as halogen: see claim 2) by reacting an arabinose donor such as Ara-U (see all examples except example 3) with the desired purine source in the presence of an enzyme capable of transarabinosylation such as *Enterobacter aerogenes* ATCC 13048 (see claim 10).
The process claimed in present application differs from D1 only in that the purine source is 2-fluoroadenine not specifically exemplified in D1, in order to give the key intermediate fludarabine which is further converted into fludarabine phosphate.

Starting from fludarabine that can be easily prepared as taught by D1, in order to provide a process for the preparation of fludarabine phosphate, is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill.

According to D2, column 6, lines 18-21, acylation may be employed as a convenient process to recover and recycle undesired N-acyl byproducts (=unfluorinated) to increase the overall yield of fludarabine from the process, The collected crude 2-fluoro 2',3',5'-tri-O-acyl compound may be purified by chromatography or recrystallization from absolute ethanol (see example 7b) and further deacetylated into pure fludarabine (see example 8a).
In order to separate fludarabine from Ara-Adenine by-products, the man skilled in the art would therefore perform an acetylation of crude fludarabine followed by a recrystallization (thus removing N-acylated Ara-A) and hydrolysis as suggested by D2, and would therefore arrive to the features of steps b) and c) without the exercise of inventive skill.

As indicated in the description on page 4, lines 26-28, the phosphorylation step d) can be performed according to any conventional technique such as disclosed in US 4357324 (= WO95/09244 = D3).

In view of D2 and D3, the skilled person would therefore regard it as a normal design option to purify and phosphorylate the crude fludarabine as obtained by the process described in D1 in order to provide a process for the preparation of fludarabine phosphate from 2-fluoroadenine.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-7 do not involve an inventive step in the sense of Article 33(3) PCT.

3) Claims 8-10:

Claims 8-10 refer to a process for the preparation of fludarabine phosphate salts with organic amines or ammonia with a high purity and to said salts.

D4 discloses a purification process of fludarabine phosphate by converting it into fludarabine phosphate lithium, sodium, potassium, calcium and magnesium salts with a purity of at least 99,5% and in a yield above 90% (see all examples) from which the subject-matter of claims 8-10 differs only in that they relate to salts with amines or ammonium.
The salts of present application however are produced in less yield and less purity than D4.

Additionally, pharmaceutically-acceptable salts of 5'-monophosphate nucleotide derivatives are conventionally chosen from alkali and alkaline earth metal ions, amines and quaternary ammonium groups (see for example D5, claim 8 and page 6, lines 13-21), so that the salts claimed in claim 10 are conventional pharmaceutical acceptable salts and in analogy to the salts of D4 can be prepared in high yield.
Consequently, no inventive step can be acknowledged to the subject-matter of claims 8-10.

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 8-10 do not involve an inventive step in the sense of Article 33(3) PCT.

4) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D2 is not mentioned in the description, nor are these documents identified therein.